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AQUATIC MESOCOSM TESTS TO SUPPORT PESTICIDE REGISTRATIONS

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INTRODUCTION

Decision makers, or risk managers, face a difficult task when making decisions concerning the registration or approval to market of pesticide products. These decisions require sound information on the potential risks resulting from pesticide uses to determine if unwanted impacts are tolerable recognizing that pesticides can benefit society. Aquatic ecosystems such as ponds, lakes, streams, and estuaries are the ultimate depositories of most outdoor-use pesticides. Aquatic ecosystems receive pesticide contamination directly from certain pesticide uses (i.e., mosquito larvicides, aquatic herbicides, etc.) and indirectly through spray drift, surface runoff and deposition of volatilized compounds from pesticide products applied to land. Risk assessments are traditionally made by combining exposure information and toxicity information to determine likelihood of an adverse effect. Exposure can be predicted by knowing the environmental fate of a compound and the use conditions, albeit not as readily as stated. Toxicity is usually predicted by employing extrapolations from single-species laboratory tests on select representative species. Although this laboratory testing has been a useful tool for risk managers, ecologists and aquatic toxicologists have recognized the weaknesses of using single-species tests alone for assessing potential ecosystem impacts (Cairns, 1981; Pimentel and Edwards, 1982; Cairns, 1984; Levin <u>et al</u>., 1984; Odum, 1984; Kimball and Levin, 1985).

Aquatic toxicologists have had limited success in obtaining predictive information from field investigations. Field testing, when used to obviate concerns of impact to aquatic organisms, can take a variety of forms. Most common are actual use field studies where representative sites such as farm ponds are exposed to typical or exaggerated (representing worst case) use practices to determine residues and/or biological effects. These actual use tests have been preferred for risk management decisions because residue or biological information collected is expected to accurately depict hazard. Since the pesticide is exposed to natural chemical, physical and biological conditions which can alter or mitigate its toxic potential even if these conditions are unknown, the actual use field test has been perceived as the best choice to unequivocally demonstrate safety. However, due to the complexity, inherent resilience and lack of replicability of these natural or semi-natural farm pond ecosystems, limited tests over one or two years may not be adequate for use in hazard assessment, especially where biological observations are limited to a few structural parameters on highly variable populations.

Parameters most often investigated in an actual use field study include survival, abundance, diversity and pathology. These parameters as generally investigated are limited to just a few of the most dominant populations. Interrelationships among several populations within a community are seldom considered. Functional parameters like production (increase in biomass/unit of area/unit of time) and assimilation (production/respiration) have been neglected.

Natural environments may not be adequately safeguarded by protecting only a few populations. Consideration needs to be given to important aspects of both ecosystem structure and function (Cairns, et al., 1972). Natural ecosystems are dynamic and cannot be effectively replicated for unequivocal cause and effect determinations, which are desired for effective risk management. Catastrophic events can usually be detected in an actual use field investigation, but subtle effects which may slowly degrade or negatively alter a system are not easily identified. Ecologists who have recognized this deficiency have developed physical models (i.e., simulated ecosystems, microcosms, mesocosms) for aquatic ecosystems which allow the necessary control and replicability to detect ecosystem-level effects (Witherspoon et al., 1976; Metcalf, 1977; Giddings, 1980). Mesocosms (experimental ponds and in situ enclosures) may offer the greatest promise for providing the requisite information for risk managers. The use of aquatic mesocosms most likely began with the experimental ponds of Swingle (1947, 1950) to determine the role of nutrient enrichment for increasing fish production, long before the term "mesocosm" came into common usage (Grice and Reeve, 1982; Odum, 1983). Several investigators have employed mesocosms for assessing effects due to chemical contaminants (Jones and Moyle, 1963; Hurlbert et al., 1972; McIntosh and Kevern, 1974; Shindler et al., 1975; Mauck et al., 1976; Menzel and Case, 1977; Tucker and Boyd, 1978; Klassen and Kadoum, 1979; Boyle, 1980; Kettle et al., 1980; Papst et al., 1980; Solomon et al., 1980; Crossland, 1982; deNoyelles et al., 1982; Giddings et al., 1984; Boyle et al., 1985; Crossland and Wolff, 1985; Kaushik et al., 1985).

Pond-like mesocosms are a good choice for investigating ecotoxicity of pesticides in aquatic ecosystems. These systems simulate ponds, shallow lakes, riverine embayments, backwaters, etc., which form habitats for many important aquatic species (Giddings and Franco, 1985). When containing assemblages of organisms together with appropriate substrates and sub-systems which are as complex as in natural communities, mesocosms should respond to chemical perturbation in a similar fashion as naturally occuring systems. As suggested by deNoyelles and Kettle (1985), "one ecosystem (the experimental pond) in the field that can be controlled, manipulated and replicated is being used to simulate the responses of another in the field that cannot (natural ponds and lakes)."

For a mesocosm study to be truly effective in supporting regulatory requirements, it must address parameters which are meaningful to risk managers. In addition, the study must be scientifically credible, performed with appropriate methods, verifiably accurate with a reasonable confidence of repeatability and applicable to predicting pesticide impacts. The purpose of this paper is to describe the criteria for an acceptable aquatic mesocosm study to be used in an ecological risk assessment of a pesticide. The rationale for these criteria will be presented as will a discussion of how the results of such studies may be interpreted. This document is not intended to detail specific test methods, but only to provide a flexible framework for developing an acceptable aquatic mesocosm study protocol.

OBJECTIVES

An aquatic mesocosm test will serve two regulatory objectives. First, it will provide a pesticide registrant supportable means for negating presumptions of unacceptable risks to aquatic organisms for their product. Such presumptions of risk are initially based on comparisons between single-species laboratory data and exposure information. Only those pesticides which are presumed hazardous to aquatic organisms from such data are required to initiate a field-level test such as an aquatic mesocosm study. The question of whether concentrations of a given pesticide, which result in adverse effects to aquatic organisms under laboratory conditions, will adversely impact aquatic organisms under field exposure conditions is addressed by the mesocosm test. And second, it will provide risk managers descriptive information on the extent of adverse impacts, both in duration and magnitude, likely to occur in aquatic systems which can then be evaluated in risk-benefit analyses.

Because an aquatic mesocosm study is an ecosystem-level test, many parameters of both ecosystem structure and function are compared between untreated and treated systems to ascertain differences. Such differences must be quantitatively and qualitatively analyzed for significance. Risk managers must know how expected exposures of a potentially hazardous pesticide impact populations, community structure or ecosystem function in a representative aquatic system before making regulatory decisions. An aquatic mesocosm study can readily address these questions.

PROPOSED DESIGN CRITERIA

Physical Description

Experimental Design --

One acceptable design is a minimum of four (4) experimental treatments consisting of a control which receives no test compound, an 'X' treatment level representing expected exposures, an 'X+' treatment level representing an upper bound, and an 'X-' treatment level representing a lower bound. At least three replicates per treatment level are minimally needed to provide the requisite resolution of effects and probability of their occurrence. However, it is recommended that the replicate number be dictated as a function of the parameters of interest and the sensitivity of their analysis.

Alternative designs which emphasize regression analysis and utilize more treatment levels with fewer or no replicates may also be appropriate. Regression designs are most useful for determining maximum exposure conditions which provide no significant impacts or a specified level of effect in test systems.

Mesocosm Number --

A minimum of twelve (12) mesocosms are required with additional mesocosms added as replicates or treatments when needed to increase the sensitivity of analysis for specific parameters.

Mesocosm Size --

Dimensions of a mesocosm must be large enough to accommodate a viable finfish population. Depth should be sufficient to provide a representative open water area, and sloped sides should provide a littoral area for macrophyte growth and finfish reproduction. An acceptable design would occupy approximately 0.1 acre surface area with a volume of at least 300 cubic meters and a maximum depth of 2 meters. Sides of the mesocosm should be sloped approximately 1 unit of drop per 2-3 units of linear distance.

Mesocosm Features --

Mesocosms can be constructed as dug-out ponds or enclosures of existing impoundments. The mesocosms should be lined with an impervious material of known adsorption for the test compound. The sediment used should be well defined and representative in composition (% clay, silt and sand; % organic carbon; % organic nitrogen; ion exchange capacity) to pond sediments in the intended use area of the pesticide. The sediment depth at the bottom of the systems should be a minimum of 15 cm. Sediments may consist of natural pond sediment or top soil. If top soil is used, the complete mesocosm should be 'seasoned' for one year prior to experimental use. This time is necessary to develop benthic biota. If pond sediments are used, a shorter 'seasoning' (e.g., 6 months) period is adequate. Organic content of the top soil should be at least 2%.

A means of interchange (circulation, fill-drain-refill, etc.) of the water between the systems during initial establishment is desirable to ensure even distribution of biota among the mesocosms. Once the systems have become established or at initiation of a test the circulation should be stopped and each system kept separate from all other systems. The required precautions to ensure no cross contamination from pond overflow during rainstorms, leakage in the circulation system, etc., should be taken from the outset.

Mesocosm Biota --

The mesocosms must contain a 'representative' pond biota. It is recommended that an established pond with diverse biota will act as a parent pond. The water in the mesocosm should be equivalent to the water of the parent pond and biota collected from the parent pond will be evenly distributed to each mesocosm to act as a starter base. Biota from other sources may be used to augment a natural assemblage to ensure adequate representation of important taxa.

Phytoplankton are expected to reach a concentration consistent with the nutrient levels of the system prior to introduction of macroinvertebrates. Nutrient levels should be within a mesotrophic classification. The macroinvertebrate fauna should include representatives of the rotifers, annelids, copepods, cladocerans, amphipods, aquatic insects and gastropods. Introduced macroinvertebrates, if necessary to augment naturally colonized populations, should not exceed 10 g wet weight/cubic meter and finfish should not be introduced at more than 2 g wet weight/cubic meter. Fish species used in the test must be of known sensitivity to the test compound (determined from acute toxicity tests) and appropriate to small pond enclosures. Finfish species used must be native North American species (bluegill sunfish alone or in combination with largemouth bass are recommended).

Mesocosm Treatment --

Treatment levels of the mesocosms will be based on exposure models and residue monitoring data if available. In a three replicate by four treatment design, the three experimental treatments will be separated into a low, intermediate and high treatment (dosed) and a control treatment (undosed). The intermediate treatment will approximate the estimated environmental concentration determined through modeling and experiential data for the intended pesticide use. It is recommended that the low treatment should be 1/10 the intermediate and the high treatment should be 10 times the intermediate. Regression designs should bracket expected exposures and expected response concentrations. Loading of pesticide into the mesocosms will be by direct overspray to simulate drift and aerial deposition and with a sediment/water slurry channeled into the system at predetermined points to simulate runoff. Model predictions with available monitoring data will dictate the timing, frequency and mode of introduction of the test material.

Measured Parameters

Chemical/Physical Properties --

Mesocosm water will be monitored for pH, temperature, transparency (turbidity), dissolved oxygen, alkalinity, total nitrogen, total phosphorus, conductivity (total hardness) and particulate and dissolved organic carbon at appropriate intervals (e.g., biweekly). Observations will be made at several locations throughout the mesocosm (which will be dictated by the physical design of the mesocosm) and at appropriate depths to allow quantification of vertical and horizontal variations. A complete water analysis should be conducted at the test initiation and termination and at significant periods during the test (i.e., pesticide inputs, substantial changes in other observed parameters, etc.). Temperature, pH and dissolved oxygen should be monitored on a continuous basis for 24 hrs. on a biweekly schedule and at significant periods during the test to provide an estimate of gross production and community respiration.

Mesocosm sediment must be analyzed for pesticide content, particle size, cation exchange capacity, organic content and pH at the initiation of the test.

Biological Structure --

Biota will be identified to species or lowest taxonomic unit practical. The schedule for sampling and collection of biological samples will depend on the design and composition of the mesocosm and must be determined prior to the initiation of the test. Collections should not be so frequent as to disrupt the system.

Phytoplankton will be collected from the water column, dominant species identified, and biomass determined by measuring chorophyll a and phaeophytin. All samples should be preserved for archival reference. Periphyton will be collected from glass slide substrates placed in the mesocosm and exposed for a minimum of 2 weeks. Periphyton will be analyzed for chorophyll a and ash free weight. Macrophytes will be identified to species, biomass determined by dry weight and per cent cover of the mesocosm determined.

Zooplankton will be collected weekly with tube cores of the water column and vertical net tows. All samples will be archived for future reference. Zooplankton samples will be analyzed biweekly by enumerating and identifying dominant species. Cladocerans should be identified to genus and differentiated by size (e.g., measured for length of muon). Macroinvertebrates, at a minimum, will be collected from emergent insect traps and artificial substrates. Sampling of sediment directly (e.g., Ekman dredge), should be employed cautiously, if necessary for tracking benthic community parameters, to minimize disruption to the benthic community. Samples will be enumerated, identified to lowest practical taxon and archived.

Finfish will be identified to species, enumerated, sexed (when possible) and measured in length and weight (wet) at introduction into the mesocosms and at test termination. Also at test termination, females will be assessed for fecundity and all collected fish will be examined for gross pathology. Spawning substrates will be placed in the systems and periodically surveyed for number of deposited eggs.

Toxicity testing and bioassays with indigenous fauna on-site and in the laboratory may be used to assist in confirming cause-and-effect relationships.

Residue Analysis --

Residues of the test material and major degradates/ metabolites will be analyzed at appropriate intervals to the environmental properties of the compound in the water, sediments and biota at a sensitivity consistent with concentrations of concern.

Meteorological Conditions --

Continuous monitoring of air temperature, wind velocity, precipitation, evaporation and solar radiation are required within 1 mile of the mesocosm test facility.

RATIONALE

Critical features of a mesocosm test design include size of the mesocosm, its composition, duration of the test and measured parameters. The test design presented is not intended to be overly restrictive, and some flexibility is allowed to adjust to specific questions of a pesticide hazard. The primary intent of these studies is to allow potential pesticide registrants an opportunity to demonstrate the environmental safety of their product under conditions closely approximating those encountered in naturally occurring systems. Risk managers require a high level of confidence that the test employed is sensitive in detecting adverse effects if they are likely to occur. Note, pesticides tested in a mesocosm study should always have demonstrated toxicity under laboratory conditions at exposure concentrations expected to be encountered under typical uses. Because of this indicated risk, the requirement for conducting these studies is justified. Registrants want to demonstrate that complex systems will mitigate the exposure or toxicity indicated from laboratory data, regulators need information on ecosystem level responses to evaluate in risk/benefit analysis.

Ecotoxicity testing with aquatic mesocosms may ideally serve both goals.

The size of an aquatic mesocosm is critical to the overall study design. Distorting influences of large predators (e.g., finfish) and an inverse relationship between mesocosm size and available surface area for periphyton growth must be balanced with informational needs and practicality for adequate replication and sampling. Finfish are important as integrators of the systems and to provide the requisite end-points for risk management decisions. The inclusion of an integrating finfish population in the systems dictates a relatively large-scale enclosure. Ponds of 300 cubic meters or larger should provide sufficient volume for reproducing populations of a species such as the bluegill sunfish, Lepomis macrochirus. Many reasons exist to recommend the bluegill as the finfish species of choice. The bluegill sunfish is a native North American species and is easily obtainable in most areas of the United States. The species is cultured throughout the country which provides easy availability of healthy stocks. In optimal conditions, the bluegill will reach reproductive maturity in 4 months (Breder and Rosen, 1966). As a preferred laboratory test species, the bluegill is considered reasonably sensitive with a large amount of toxicology data available for interpretational and comparative purposes. Also, a great deal is known about its biological requirements in small impoundments. Finally, and more importantly, the bluegill is planktivorous as a juvenile and insectivorous as an adult allowing a single finfish species to cover two important trophic roles. Where finfish predator/prey relationships are important, piscivorous species (e.g., largemouth bass, Micropterus salmoides) may be included in systems of 1000 cubic meters or larger. Although smaller systems could support the piscivorous trophic level, the large systems will provide larger populations which should allow more precise tracking of system perturbations.

The composition of the experimental mesocosms should include naturally derived biota, organisms obtained from established natural systems free from chemical contamination. It is assumed that a natural asssemblage from most origins would contain a collection of organisms diverse and complex enough to adequately represent pond-like systems. Pond flora and invertebrate fauna are relatively consistent from one area to the other at gross taxonomic levels, and differences at the species level may be trivial where genera and families are represented. Finfish species other than the bluegill should be chosen for specific purposes, and should include species appropriate for the confines of a mesocosm and with known sensitivities for the test compound.

The duration of the test depends in part on the environmental half-life of the test compound and its chronicity. It is expected that, ponds can be established in early spring or the preceding fall or winter, treated at representative times in the year corresponding to the expected use, as indicated by exposure models, and terminated in late fall or early winter. When treatment timing does not allow determination of effects on finfish reproduction or if system recovery is of interest, studies may have to be continued over winter and through an additional season. Treatments in the second season are optional depending on the registration intent and could be required if the temporal dynamics of the pesticide in the system warrant. Tests conducted in outdoor ponds must have at least 1/3 of the pond area greater than 1.5 meters in depth if the test is continued over winter to prevent complete freezing.

The parameters chosen for measurement are considered minimal for investigating structure and function of the systems. A biweekly sampling regime is considered adequate for tracking potential perturbations except for zooplankton and finfish. Zooplankton are expected to serve as sentinels of system distress and should be sampled weekly. It is sufficent to analyze zooplankton data biweekly, but weekly samples should be collected and archived since they may be required to explain disturbances if these occur. Finfish in these relatively small systems cannot be routinely sampled and since the summation of their production is an important end-point, sampling of the finfish population should occur only at test termination. The intent of a mesocosm test is to determine how a contaminant perturbs an aquatic system and the trajectory, magnitude and duration of the peturbation if it occurs. Protocols specific to a pesticide of concern should be discussed with the Environmental Protection Agency on a case-by-case basis when the study will be used to support a product registration.

The experimental design of four (4) treatment groups including controls with three (3) replicates in each group was chosen for several reasons. Successful studies by deNoyelles _et al_. (1982) and Boyle _et al_. (1985) utilized this same general design. Differences in treatment groups as little as 15% from controls for fish survival and recruitment were detected as significant at p=0.0001. Also, twelve (12) mesocosms 0.1 acre in size appear to be logistically manageable for the observational requirements associated in a test. The design additionally allows the expected exposures resulting from the pesticide use to be bounded by higher and lower treatment groups.

INTERPRETATION OF RESULTS

The objective interpretation by qualitative and quantitative methods is defined here as analysis. Subjective interpretation and regulatory implications will be termed evaluation. Analysis may unequivocally demonstrate a change in a treatment pond in comparison to control yet evaluation could determine such change to be trivial. Risk management decisions involve much more than simple analysis and evaluation of ecotoxicity tests such as economic and/or social considerations, but such tests could contribute strongly to the ultimate decisions.

A mesocosm test involves many levels of biological organization and effects at any and all of these levels will be analyzed and evaluated for significance. Ecosystem stress is manifest through changes in nutrient cycling, productivity, the size of dominant species, species diversity and a shift in species dominance to opportunistic shorter-lived forms (Rapport et al., 1985). Harte et al. (1981) identify direct chemical threats to drinking water quality, impairment of sports-fish populations, aesthetic loss from increasing turbidity or eutrophication, enhanced odor-producing biological activity and increased likelihood of disease-bearing vectors and pathogens as the water quality issues of most concern to the public, and these intuitively would be of most concern to risk managers. Of these, impairment of sports-fish populations would be a very important attribute to be affected, since it is the one most likely to express an unwanted change in any other attribute as well. Therefore, interpretation of ecotoxicity tests with aquatic mesocosms will have a bottom-line assessment of the potential for adverse effects to finfish populations. Finfish occupy the higher trophic levels of aquatic ecosystems and therefore are the summation of much of the activity at lower levels. Impacts at lower levels should be expressed at all higher levels which are dependent upon them. This is not to imply that impacts to invertebrates or phytoplankton are not important, just that effects at the lower levels will be interpreted for their impact to the finfish level. Notable exceptions would include pesticide uses likely to expose habitat of commercial shellfish importance or where endangered/threatened aquatic species are present. In any event, effects on organisms other than finfish will be included in an ecological risk assessment for completeness and to keep risk managers informed of all effects which may result from a pesticide residue in aquatic environments.

To better exemplify the above discussion, consider two responses which may occur in a mesocosm test. In one, fish production is demonstrably reduced by treatment, while in the other, fish production is unchanged between control and dosed systems. If in the first instance, transient reductions to macroinvertebrates were responsible for the fish production decrease and the decrease is assessed as temporary, then such an impact may be considered minor. On the other hand, if the second response is the result of permanent changes in another component (e.g., zooplankton) of the system which will not allow fish production to be sustained, then such a response may be considered major. In both cases, fish production parameters were used in making an assessment of the responses and, in both cases, other system component parameters were necessary to interpret the findings.

Three general outcomes of a mesocosm treatment level are possible: (1) no discernible effect to any measured parameter; (2) a marginal effect to one or more parameters such that the system can compensate for the perturbation and fully recover, and there is no substantial alteration seen or expected for finfish populations; and (3) a substantial effect to one or more parameters such that the system cannot accommodate the stress and fails to recover or finfish populations are markedly reduced. These three outcomes are possible in each of three treatment levels. By bounding an intermediate level approximating expected exposure concentrations with a lower and higher level by an order of magnitude, evaluation of the results allows some inference of probability for its occurrence. For outcome (1) at all treatment levels, environmental concerns are sufficiently obviated to allow pesticide registration to proceed accordingly. For outcome (3) at all treatment levels, serious hazard is indicated and registration is warranted only with substantial benefits and heavy use restrictions. For other outcomes in between these extremes, registrations will depend on risk/benefit analysis.

Registrations could be allowed to proceed where only marginal impacts are observed at the low and intermediate levels, but a condition of field testing/monitoring may be imposed for the initial years of the registration to ensure no unreasonable impacts occur. Additionally, where marginal effects are suggestive of high risk then additional testing (~~field~~ field and/or laboratory) may be needed prior to registration.

The power of the mesocosm experimental design is expected to be greater than the statistical sensitivity of hypothesis testing such as ANOVA would initially suggest. The design is somewhat constrained by practical limitations such that, at a minimum, only twelve mesocosms are required for conducting the test. By bounding an intermediate exposure level with a high and low level additional statistical strength can be gained by way of regression analysis where appropriate. From a regulatory viewpoint, if there is no discernible effect either qualitatively (e.g., change in dominant species) or statistically (e.g., no significant difference in an observational parameter at a confidence level appropriate to that parameter) between the high treatment, which represents an exposure concentration 10 times greater than generally expected under actual use conditions, and control systems, given that the systems are representative of aquatic ecosystems, then one can be reasonably confident that the product tested will not threaten aquatic resources.

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APPENDIX

AQUATIC MESOCOSM WORKSHOP

A two-day workshop was held April 8 and 9, 1986 at George Mason University in Fairfax, Virginia. Participants at the workshop were from EPA, academia and the National Agricultural Chemicals Association. Organization of the workshop was such that participants were subdivided into four working groups of approximately ten members.. Each working group addressed technical questions related to mesocosm testing in only one of four areas -- 1) mesocosm size and composition, 2) observational parameters, 3) treatment, replication and duration, and 4) interpretation or results. All participants attended a general discussion on the last day which culminated the workshop. Recommendations from participants were taken from the summaries of the working group chairmen and from specific comments on a circulated draft mesocosm test document. The summaries from the working group chairmen are as follows:

Working Group 1 -- Mesocosm Size and Composition

1. Finfish should be included in the mesocosm experimental design. Fish are needed to evaluate potential adverse effects at that trophic level. Fish are needed as a component of the mesocosm system to complete the model ecosystem design and, thus, allow the development of potential secondary effects due to the presence of a consumer species.

2. Finfish in the mesocosm should be planktivorous as juveniles and insectivorous as adults. Top predators require too large an enclosure and too long a time to develop a stable population.

3. Two size classes of finfish should be introduced -- young, sexually mature fish which will reproduce during the experimental period and immature fish. Two size classes provide a minimal model of natural finfish population distribution. Mature fish can be used within one annual season for reproduction evaluation.

4. Population density of the finfish should be adjusted to simulate natural population densities for the chosen fish species in a similar (to the mesocosm) habitat. Densities within the range of 2-5 g/cubic meter are appropriate. Care must be taken due to potential serious disruption of individual systems if loss of fish occurs. Sufficient numbers of fish are needed to reduce effects of small losses.

5. Volume of the mesocosm should be at least 300 cubic meters. Maximum depth should be 2 meters. Sides of the mesocosm should be sloped approximately 1 unit of drop per 2-3 units of linear distance. Dimensions must be large enough to accomodate a reliable finfish population. Depth should be sufficient to provide a representative open water area, and sloped sides should provide a littoral area for macrophyte growth and finfish reproduction. The design described should occupy approximately 0.1 acre surface area.

6. Mesocosm structure should normally be a dug pond, but naturally occurring or 'old' ponds which meet all other criteria will not be excluded. The required size and shape is not amenable to prefabricated, container construction. Provided sufficient land is available, the mesocosms can be machine dug at relatively low cost.

7. Larger ponds may be subdivided to act as replicate mesocosms provided that each subdivision meets all other criteria. Subdivisions should all be to the same size, shape and dimensions. Design of subdivisions should be such that no leakage occurs between adjacent mesocosms. Materials used for subdivision should not introduce any toxic substance which could significantly affect the test.

8. Bottoms of the dug ponds should be lined with impervious material (clay or plastic sheet) to avoid interconnection with groundwater.

9. Sediments should be added (over the impervious layer) to a depth of at least 10 cm. Sediments may consist of natural or aged pond sediment or top soil. If top soil is used the complete mesocosm should be 'seasoned' for one year prior to experimental use. This time is necessary to develop benthic biota. If pond sediments are used, a shorter 'seasoning' (e.g., 6 months) period is adequate. Organic content of the top soil should be at least 10%.

10. Ponds may be reused without sediment removal if water quality parameters and biota are shown to be similar among all mesocosms. Sediment removal and/or other cleaning to assure uniform starting conditions may be carried out as needed.

11. Sampling methods should not significantly disturb the sediments or water column.

12. An even distribution of biota among all test mesocosms is an overriding objective. Various options may be employed to achieve this goal. For example, all mesocosms could be drained and refilled from a common source immediately before the start of a test cycle, or a mixing system among all mesocosms may be employed provided an even distribution of the biota can be achieved.

13. Establishment of a 'representative' pond biota is a major objective. This may be achieved by addition of biota from other sources including natural ponds, aged systems or cultured material.

14. Treatment time should coincide with applicable use patterns. If established 'aged' pond water is used to fill the mesocosms, they should be filled and sampled for at least 4 weeks prior to introduction of the test compound. If water from another source is used, sufficient time must be allowed for development of a representative pond flora and fauna.

15. All mesocosms should contain representative macrophytes evenly distributed with regard to biomass and species composition. At least submerged macrophytes should be represented. Prior to the start of an experiment, macrophyte population structure should be mechanically adjusted to meet the above criteria. Macrophyte populations should not be manipulated during the experimental period. Mesocosm design criteria are intended to provide some areas which are macrophyte free.

16. Minimal fertilizer addition can be used to avoid a pond model which would be highly oligotrophic. This item is not to be interpreted to mean that the mesocosm should be eutrophic. The objective is to model a 'representative' biologically active ecosystem.

17. Water level within the mesocosm systems may be adjusted to account for evaporation or rainfall inputs. If the levels change from the starting conditions by 5 cm either over or under, they should be adjusted to the initial levels. Water added to the systems during the experiment should be filtered through a sand filter (e.g., swimming pool type filter) to remove most plankton. All experimental mesocosms should be adjusted to the same level simultaneously. Particular care must be taken in subdivided systems

curtained with flexible materials so that water levels in adjacent enclosures remain the same.

18. It is suggested that a mesocosm test in support of the registration of a particular compound be conducted in the major use region for that compound.

19. Because of the experimental design and controlled nature of the experiments it is felt that results from a test in one geographic region could be extrapolated to other regions.

20. One set (multitreatment, replicate mesocosm) ~~of~~ mesocosm tests could be sufficient for regulatory purposes, either acceptance for registration or denial of registration. Should the result imply significant, long-term hazard in one geographical location further mesocosm testing in other areas under different conditions could be used to achieve limited compound registration for similar regions.

Working Group 2 -- Observational Parameters

1. Physico-chemical parameters

A. Water column

	Parameter	Frequency	Location of sample
1)	pH transparency alkalinity total nitrogen total phosphorus conductivity or total hardness dissolved organic carbon particulate organic carbon	biweekly	integrated sample (from sufficient samples to include vertical and horizontal variations)
2)	dissolved oxygen temperature pH	biweekly	subsurface and 10 cm above the bottom at sufficient points to include all variations needed to characterize the mesocosm
3)	temperature	biweekly (maximum and minimum for 2 weeks prior)	0.5 m below the surface

4)	target pesticide	at regular intervals appropriate to the compound
5)	contaminants	beginning and end of test

Considered but rejected: BOD, COD, free CO_2, redox potential, forms of N, forms of P, sulfur (except in cases involving arsenicals), and inorganic carbon.

B. Sediment

	Parameter	Frequency	Location of sample
1)	particle size cation exchange capacity organic content pH pesticide scan	at beginning of test	
2)	target pesticide	at regular intervals appropriate to the compound	

Considered but rejected: dissolved oxygen, depth of redox potential discontinuity (RPD) layer, oxygen uptake, ammonia efflux, sulfur, temperature.

C. Atmosphere

Parameter	Frequency	Location of sample
air temperature wind velocity precipitation evaporation solar radiation	continuously	at a meteorological station within 1 mile of the mesocosm

2. Biota

A.	Biotic component	Frequency and Technique of Sampling	Parameter Measured
1)	Phytoplankton	biweekly measurements from at least 3 integrated samples	chlorophyll a and phaeophytin; identification of dominant species; preserve all samples for archival reference

2)	Periphyton	biweekly collections of glass slide substrates exposed for 2 weeks	chlorophyll a and ash free weight
3)	Macrophytes	sampling as appropriate and at end of test	percentage cover of each species; dry weight
4)	Zooplankton	weekly collections, biweekly counts; tube cores of the water column and vertical net tows	archive all samples; in alternate weeks count dominants to species or genus and note length of muon of cladocerans
5)	Macroinvertebrates	biweekly collections from emergent insect traps and artificial substrates	count to lowest practical taxon; archive all samples
6)	Finfish	sampling at beginning and end of test (test should be long enough to span reproductive cycle)	count to species, measure length & wet weight; note pathologic conditions; target pesticide concentration in fish tissue

Considered but rejected: sampling of microbiota and epipelon.

B. Bioassays

Bioassays were considered using caged organisms in the mesocosm or toxicity testing of organisms removed from the mesocosm, but both were rejected as being redundant.

C. Ecosystem function

While these measures (production at each trophic level) were thought to be valuable, it was felt that the measurments of structure of the mesocosm ecosystem over time were sufficient to determine these parameters.

Working Group 3 -- Treatment, Duration and Experimental Design

1. A minimum of 4 treatments consisting of a control, an 'X' treatment level representing expected exposures, an 'X+' representing an upper bound, and an 'X-' representing a lower bound. Expected exposures would be predicted from appropriate drift and runoff models and/or relevant empirical studies.

2. A minimum of 3 - 5 replicates per treatment as a function of parameters of interest, mesocosm design, etc. Existing data sets (and new data as it becomes available) should be consulted for establishing minimum replicate numbers.

3. Treatment frequency is based on the number of applications permitted on the pesticide label for aerial drift simulation and on established runoff models (e.g., SWRRB) for runoff simulation.

4. Aerial treatment simulations should be by spray on surface with pesticide finish spray. Runoff simulations may be by uniformly distributing a subsurface slurry of pesticide in water and sediment. Problems are expected in determining the method for creating the slurry, dealing with compound alterations while on soil, determining the appropriate particle size, and so on.

5. Test ponds must be aged at least one year if using established pond sediments. Use of established pond sediments in mesocosms is a trade off between pond stability and similarity of replicates. Test ponds using topsoil will require study to determine an adequate pretreatment interval. The duration of a mesocosm test is at least 1 full growing season and longer for persistent compounds, but not without added problems.

6. Macrophytes will cause increased heterogeneity and variance between replicates in addition to other sampling problems. No macrophytes or intensely controlled macrophytes should be used in the test ponds. Two species of fish (i.e., bluegill sunfish and largemouth bass) are recommended rather than two age classes of one species. The ponds should have fish, sediment, invertebrates and other orgainisms seeded from an established pond.

Working Group 4 -- Interpretation of Results

1. Finfish are of primary importance for detrmining significant effects and meaningful endpoints from conduct of a mesocosm study. Fish number, biomass, growth, fecundity (estimated from nesting substrates), survival, and residues in tissues (e.g., gravid gonads) should be determined.

2. Zooplankton community structure determined by observations on total biomass and species dominance. Size may be used instead of detailed taxonomy. Changes to zooplankton should provide the first indications of system impact.

3. Benthic community structure should be studied to determine major taxonomic shifts. Such shifts will give clues on changes of detrital processes.

4. Plankton and periphyton should be studied for changes in chloropyll a and dominance over time.

5. Community metabolism is tracked by monitoring pH, dark respiration and light oxygen evolution, preferably by automated continuous recorders.

6. The use of diversity or absolute ratio indices should be avoided.

7. Geographic extrapolations of mesocosm results may be derived from available fate models. Biological extrapolations from mesocosm investigations are limited without linking field investigations.

8. Fish are the integrators of the experimental ecosystems. The systems should not be overloaded by fish biomass and the fish should not require external feeding.

9. Sediment is expected to be somewhat of a "black hole" for certain compounds. The sediment requires characterization for the sorption nature, rates, etc. The biomass/sediment ratio should be considered an important design parameter.

10. Bioassays with selscted organisms are important before, during and after treatment of the mesocosms.

11. In the pesticide registration and hazard evaluation process, there is a continued role for microcosm and full-scale field investigations.

Substantive comments received from participants of the April, 1986 workshop not fully resolved within the document are discussed here:

1. A strong reservation exists among some that reliance on fish data may be precipitous due to inherent variability expected between ponds. It is suggested that credibility of an approach which relies on fish population parameters would be enhanced by referencing successful studies and detailing the statistical differences, specifically if three replicates would be sufficient to separate treatment differences. It is further suggested that such data could be used in defining performance criteria for use in evaluating the acceptability of tests dependent on these parameters.

Comment -- The Agency agrees that documentation of successful studies is needed to allow implementation of the mesocosm test philosophy without reservation. However, as a new initiative in this arena few mesocosm studies with pesticides exist and none of these tests are fully consistent with the proposed design. Because of this lack of empirical data to support the proposed design, the criteria presented here and Agency position are that this document represents only a proposed framework for fulfilling aquatic organism simulated and/or actual field test requirements in support of pesticide registrations. The Agency is convinced that the proposed design adequately represents a consensus among the scientific community, that reliance on fish data are consistent with the available data base, and the Agency fully expects tests conducted in accordance with these criteria will allow acceptable hazard evaluations of pesticide registrations to aquatic ecosystems. It is anticipated that as an empirical base of these tests is developed the Agency will be in a position to evaluate its position and further specify the limits of acceptability for these tests. In the interim, the Agency will use the criteria presented as guidance in evaluating simulated aquatic field investigations.

2. Some confusion is still present on how an Estimated Exposure Concentration (EEC) will be used in specifying treatment levels.

Comment -- An EEC is used when empirical data on expected exposure concentrations are absent or insufficient for specifying treatment levels. Agency calculated EECs are based on empiricaly derived models and manipulated over a range of likely scenarios. Treatment levels should be designed to span this range such that a low level would be at or below the lowest expected exposure concentration, a high level at or above the maximum or worst-case exposure concentration and a median level which roughly approximates a typical exposure concentration consistent with the supported pesticide label. The exposures in the test systems should simulate as nearly as practical the modelled exposure. That is, the water column concentration in the test system should be equivalent to the modelled water column concentration for that treatment level, the sediment-bound concentration in the test system should be equivalent to the modelled sediment-bound concentration for that treatment level, etc. Therefore, sufficient chemical needs to be added to a test system to ensure that the water concentration is equivalent to the modelled value after chemico-physical partitioning. In general, order of magnitude differences between treatment levels in a '3-dose by 3-rep' mesocosm test design will suffice, such that a median treatment level (x) is bracketed by a low level (1/10 x) and a high level (10 x). Again, the median level represents a typcial exposure condition expected for the given pesticide use under investigation.

3. Several participants have suggested experimental design changes to provide better dose-response information and thereby improve result interpretation (i.e., '5-dose by 2 rep','11-dose by no rep', etc.).

Comment -- The Agency has no objections to modifications in experimental design so long as a minimum of 12 mesocosms are utilized and the minimum and maximum expected exposures are bounded by dose levels employed. A preference is given to a '3-dose by 3-rep' design because, as the Agency believes, it can effectively span the expected exposure range and provide adequate replication to separate obfuscating observations which result from individual differences between the mesocosms. The Agency will rely solely on established differences in interpreting the results from these tests and true effects which go undetected would, therefore, be considered to pose minimal threat to aquatic ecosystems. Designs with less than 3-reps/level may not provide equivalent separation.

4. A few participants objected to size limitations of the mesocosm and preference given to bluegills as the fish component. It is felt that other fish species or even invertebrate species could provide the requisite information and certain of these species could be tested in systems of less than the 300 cubic meters.

Comment -- The Agency dependence of native North American species for use in performing ecological effects studies has been well established. Mesocosm tests must include finfish for both direct and indirect effect evaluations. Effects seen on invertebrates alone cannot provide these data. Direct responses to a toxicant may not be fully exhibited by finfish in the laboratory. Alterations of behavior, for example, could affect feeding, reproduction, etc., in the field which would not be easily discernible from laboratory data. Indirect responses of finfish to a toxicant may not be explained by changes in invertebrate production alone. Alterations of diet, for example, could impact a fish differently when it is stressed by a pesticide. Bluegills are perceived as the most appropriate species when the mesocosm includes only a single fish species. The same rationale as used for preferring it in acute laboratory tests can be given together with its life history (planktivore/insectivore and reproductive maturation in as little as 4 months) and extensive culture experience in small ponds. Many of the mesocosm studies referenced successfully included bluegills. Certainly other species of fish may be used but the Agency would be hesitant to approve protocols with different species without sufficient justification. Since bluegills are preferred, 300 cubic meter systems would be the minimally accepted mesocosm size consistent with the species requirements.

5. Some participants felt that the list of measured parameters were too extensive and emphasized system structure without consideration of system function.

Comment -- The list of recommended parameters to be measured during a mesocosm test is almost exactly those recommended by the sub-group dealing on the issue at the April workshop. This list was pared down from a more extensive list and determined to be the minimum data set sufficiently comprehensive to charaterize pesticide effects. Additional emphasis may be needed for some chemicals and can only be determined after scrutiny of their database. The sub-group also determined that the listed measures of structure over time can provide sufficient information on ecosystem function to draw meaningful conclusions.

6. A few participants commented on macroinvertebrate sampling and stocking in the test mesocosms. It is felt that insufficient emphasis is given to benthic invertebrates and aquatic insects.

Comment -- Potential disruption of benthic sediments is the overriding consideration in limiting macroinvertebrate sampling to artificial substrates and emergent traps. However, this limitation is a "minimal" limitation in that additional sampling of system substrate and shoreline is acceptable. Note that increased sampling of the systems will follow the law of diminishing returns. As more sampling takes place the greater the likelihood that the system will be disrupted. The point was well taken by some that stocking of aquatic insects into the systems may be wasted effort as natural colonization should be more than adequate. The Agency intent here was to ensure a reasonably representative macroinvertebrate fauna in systems which may have had limited time for natural development.

7. Some confusion exists on whether mesocosm tests are designed to evaluate ecosystem effects or to evaluate indirect effects on fish.

Comment -- The Agency wants to emphasize that the purpose of the mesocosm test philosophy is to evaluate ecosystem effects. However, interpretations of the significance, if any, of these effects will be directed towards socially meaningful ends (e.g., finfish). Finfish parameters are perceived as the ones which will be less variable and an integration (for some species of fish) of other system parameters, therefore more sensitive from an analysis viewpoint. The Agency understands that other ecosystem attributes are important and evaluations will include all such attributes impacted. Pragmatically, effects to finfish will, in most instances, carry the overriding emphasis of potential regulatory outcomes.